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Sugar Binding Properties of Various Metal Ion Induced Conformations in Concanavalin A†

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ABSTRACT: Concanavalin A is known to undergo a first-order conformational transition when metals are added to the demetallized protein at pH 5.6 (Brown, R. D., III, et al. (1977) *Biochemistry 16,* 3883–3896). The rate constants for this process, which we have measured using a polarographic technique, are identical when zinc, cobalt, or manganese occupies S1 and calcium occupies S2. The reducible sugar, *p*-nitrophenyl α-D-mannopyranoside, binds only to the locked conformational structure which is formed upon the addition of metals. The affinity of the protein for sugars is dependent upon occupancy of S1 and S2 and quite sensitive to the identity of the metal in S2. The metals may be removed from the locked protein structure and the protein temporarily retains its ability to bind with sugars but with a considerably lower affinity. The locked form of concanavalin A is unstable at a pH near 2 and unfolds to the unlocked structure with a half-life of 25 min resulting in simultaneous loss of metal and sugar binding.

The sugar binding and cell agglutination properties of concanavalin A[1] (Con A) are thought to be mediated by specific metal ions. The native crystalline protein contains one divalent manganese and one divalent calcium ion per protein monomer located near the established sugar binding region (Brewer et al., 1973; Hardman & Ainsworth, 1973; Villafranca & Viola, 1974). The function of these metal ions appears to relate to the stabilization of a polypeptide loop on the surface of the molecule to form the specific sugar recognition site. Thus, these metals must be added to apo-Con A before the protein will bind with simple monosaccharides and dextrans or agglutinate whole cells. Recently, however, other investigators have questioned the calcium requirement in maintaining the structural integrity of the active protein. Brewer et al. (1974) have presented evidence that calcium may be dialyzed away from the intact manganese-calcium-Con A ternary complex leaving a calcium deficient protein which retains all properties of the native system while Grimaldi & Sykes (1975) have suggested that calcium plays a catalytic role in the conversion of the apo-protein conformation to an active sugar binding conformational state. Results from various experiments in our laboratory did not agree with these earlier reports and have led us to search for a convenient method to examine more definitively the function played by the metals in establishing the sugar recognition site in Con A. Brown et al. (1977) have recently presented a comprehensive examination of the different conformational states of Con A by following the time and frequency dependence of the proton relaxation rates of the divalent manganese derivatives. They show Mn^{2+} and Ca^{2+} form a metastable ternary complex with apo-Con A which subsequently undergoes a first-order transition to a more stable ternary complex. The latter ternary complex binds calcium so tenaciously that it takes days to dialyze away the bound Ca^{2+}.

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[1] Abbreviations used: Con A, concanavalin A; EDTA, ethylenediaminetetraacetate; PNPM, *p*-nitrophenyl α-D-mannopyranoside; DME, dropping mercury electrode; ESR, electron spin resonance; P and PL refer to the "unlocked" and "locked" conformations of Con A, respectively (see Brown et al., 1977); C, divalent calcium; M, divalent manganese (or any other divalent first-row transition metal ion where noted).

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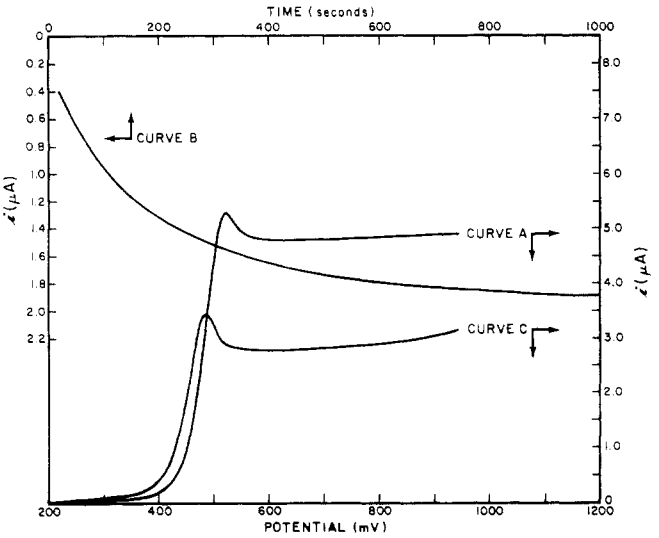

FIGURE 1: Polarographic reduction of *p*-nitrophenyl α-D-mannopyranoside at 20 °C in 0.1 M sodium acetate, 0.3 M sodium chloride buffer at pH 5.6. (Curve A) Current vs. potential scan for a solution containing 300 μM PNPM and 200 μM apoconcanavalin A. (Curve B) Current vs. time plot (at a constant −700 mV) for solution A upon the addition of 1.6 mM zinc and 1.6 mM calcium ions. (Curve C) Current vs. potential scan for final equilibrium mixture.

These investigators also reported that the same locked protein conformation could be attained upon introduction of Mn^{2+} into both the normal transition metal site (S1) and the calcium site (S2). Furthermore, removal of excess Mn^{2+} resulted in a retainment of the locked protein conformation for several days at 5 °C.

We report here the results of our examinations of the sugar binding properties of these various conformations of Con A and the kinetics of the conformational transitions which occur upon the binding of metal ions.

Materials and Methods

Con A was isolated from jack bean meal by the method of Agrawal & Goldstein (1967) and purified of its fragmented subunits according to Cunningham et al. (1972). The purified product, homogeneous by sodium dodecyl sulfate gel electrophoresis, was demetallized by dialyzing against 50 mM EDTA at pH 3. The apo-Con A was finally dialyzed against a buffer solution containing 0.1 M sodium acetate and 0.3 M sodium chloride at pH 5.6 and concentrated to approximately 50 mg/mL. The protein concentration was determined spectrophotometrically in this buffer at 280 nm using the extinction coefficient, $E_{1cm}^{1\%} = 12.4$ (Kalb & Levitzki, 1968).

Stock metal solutions were prepared from the chlorides into the same pH 5.6 buffer. *p*-Nitrophenyl α-D-mannopyranoside (PNPM) was purchased from Sigma and its concentration determined spectrophotometrically using $\epsilon = 10\,000$ cm^{-1} M^{-1} at 305 nm (Hassing & Goldstein, 1970).

Polarograms were recorded automatically using a Parr Model 174A Polarograph at a dropping mercury electrode (DME). PNPM displays a one electron, reversible reduction wave with an $E_{1/2} = 450$ mV referenced to a saturated calomel electrode. The exact half-wave potential is somewhat sensitive to solution composition due to mercury adsorption which results in maxima in the polarograms. The limiting diffusion current, i_d, cathodic at 600 mV was linearly proportional to the concentration of PNPM both in the absence of Con A and in the presence of apo-Con A. The contribution of Con A bound PNPM to the diffusion current at −700 mV was negligible compared with free PNPM and thus the measured diffusion current is directly proportional to the free PNPM concentration. The polarographic cell had a minimum solution volume of 2.8 mL and was thermostated (±0.2 °C) with a Haake external water circulator. All solutions were deaerated immediately preceding polarographic analysis and a steady stream of nitrogen was passed over the solution during polarographic scans or when making further additions to the solution. The electron spin resonance experiments were performed on a Varian E4 spectrometer at room temperature.

Results

Curves A and C in Figure 1 are representative of pulse polarogram traces of PNPM solutions containing 200 μM apo-Con A in the absence and presence of excess divalent metals, respectively. The decrease in i_d at −700 mV provides a direct measure of the free PNPM in the solution containing the metallized derivative. Curve B represents a trace of this decrease in current as a function of time after the addition of 1.6 mM Zn^{2+} and 1.6 mM Ca^{2+} to the apo-Con A–PNPM mixture. The rate of diffusion current decrease was first order in Con A concentration and zero order in PNPM and metal concentrations in solutions containing a fivefold or greater excess of metals over Con A.

Following the conventions adopted by Brown et al. (1977), these time dependent changes may be described by three equilibria.

$$M + C + P \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} CMP \quad (1)$$

$$CMP \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} CMPL \quad (2)$$

$$CMPL + S \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} CMPLS \quad (3)$$

CMP represents the metastable ternary protein complex containing a divalent transition metal, M, and a divalent calcium ion, C. This conformation of the protein which fails to bind with sugars, S, was previously labeled the "unlocked" conformation (Brown et al., 1977). CMP undergoes a slow (rate determining in the above equilibria) structural change to the more stable "locked" conformation, CMPL. This form of the protein now contains the specific sugar recognition site and rapidly reaches equilibrium with S to form the quaternary CMPLS complex. Brown et al. (1977) have shown that Mn^{2+} and Ca^{2+} are in rapid equilibrium with P to form CMP while these same two metals dissociate from the locked, CMPL, complex with a time constant of days. The equilibrium represented by k_2/k_{-2} lies far to the right and thus the conversion of CMPL to CMP is negligible during the time course of these experiments. The dissociation of C and M from CMPL has been previously shown to be small (Sherry et al., 1975; Brown et al., 1977). The on-off rate of PNPM with CMPL is also rapid (Lewis et al., 1976) in comparison with the time course changes we observe in Figure 1. Thus, the decrease in diffusion current traced in Figure 1 (curve B) is a direct measure of the first-order rate constant, k_2, for the conformational transition from CMP to CMPL.

The CMP → CMPL conversion does not take place in the presence of Zn^{2+} or Co^{2+} alone even after incubation of the apoprotein with an excess of these single metals for days at room temperature. The effect of added cadmium or lead could not be examined using this technique due to an interference by the normal reduction waves of these metals at the DME. No interference was observed for Zn^{2+}, Co^{2+}, Ca^{2+}, or Mn^{2+}. The temperature dependence of the first-order rate constants (k_2) for these metals and the corresponding PNPM + CMPL

TABLE I: First-Order Rate Constants for Conversion of Concanavalin A from an Inactive Conformation to the Active Sugar Binding Conformation (CMP→CMPL) and the Association Constants for *p*-Nitrophenyl α-D-Mannopyranoside Binding to the Active Conformation (CMPL)[a]

Temp (±0.2 °C)	$k_2 \times 10^3$ (s^{-1})	$k_3/k_{-3} \times 10^{-4}$ (M^{-1})
10	1.0 ± 0.1	1.33 ± 0.12
15	2.1 ± 0.1	1.00 ± 0.09
20	4.9 ± 0.2	0.82 ± 0.12
25	9.5 ± 0.4	0.64 ± 0.05
30	23 ± 1	0.52 ± 0.03
35	34 ± 2	0.38 ± 0.05

[a] The rate constants and equilibrium constants were measured upon addition of a fivefold molar excess of Ca^{2+} and Zn^{2+}, Co^{2+}, or Mn^{2+} to apo-Con A in 0.1 M sodium acetate buffer at pH 5.6. The reported errors are the standard deviation in 8–20 determinations at each temperature.

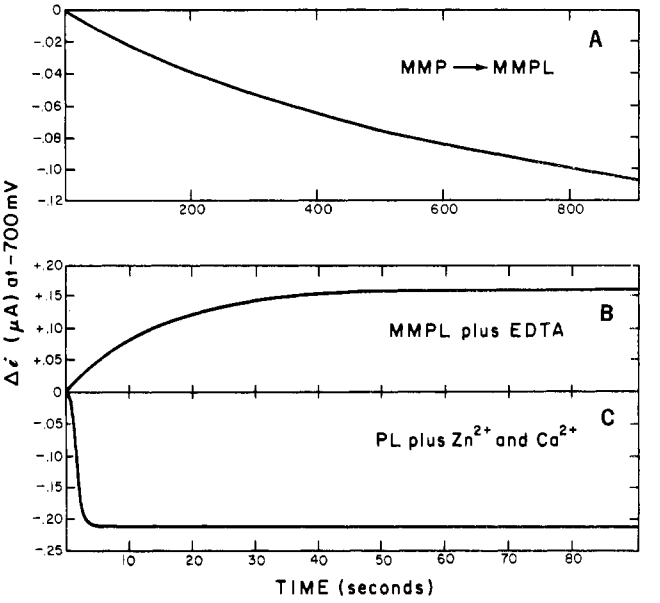

FIGURE 2: Current vs. time plots at a constant cathodic potential of 700 mV and 35 °C. (A) Decrease in current upon addition of 5.88 mM manganese to a solution containing 217 μM apoconcanavalin A and 27.5 μM PNPM in 0.1 M sodium acetate, 0.3 M sodium chloride at pH 5.6. (B) Increase in current upon the addition of 11.3 mM EDTA to the equilibrium mixture in A. (C) Decrease in current upon addition of 11.0 mM zinc and calcium to equilibrium mixture in B.

equilibrium constants are presented in Table I. The results indicate the values of k_2 and the protein-sugar association constants are insensitive to the identity of M in S1 (Mn^{2+}, Zn^{2+}, or Co^{2+}) in the presence of excess Ca^{2+}.

Manganese appears unique among the divalent transition metal ions in its ability to initiate the conversion of CMP to CMPL without the addition of calcium. Excess Mn^{2+} added to apo-Con A results in a slow conversion of the protein to a conformation which again binds sugars. Brown et al. (1977) have shown that Mn^{2+} binds in the calcium site (S2) in addition to the normal transition metal site (S1) and slowly produces the same "unlocked" to "locked" conformational change (MMP to MMPL). Interestingly, the Mn^{2+} ions were found quite labile in MMPL in comparison with CMPL and could readily be removed with EDTA leaving the apo-locked conformation, PL. This offered us an opportunity to test the absolute metal requirements for sugar binding to the protein. The conversion of Con A from P to PL is considerably slower in the presence of excess Mn^{2+} alone than is the corresponding rate in the presence of Mn^{2+} plus Ca^{2+}. The first-order rate constant, k_2', for the conversion of MMP to MMPL is 0.75 ± 0.02 × 10^{-3} s^{-1} at 35 °C (Figure 2A), a factor of 45 slower than the corresponding rate of CMP to CMPL conversion. This same rate constant was obtained for solutions containing 11:1 and 22:1 mol ratios of Mn^{2+}:monomeric Con A indicating that both S1 and S2 are saturated with Mn^{2+} under the conditions of these experiments. The locked form of Con A containing Mn^{2+} in both S1 and S2 also displays a lower affinity for PNPM than does CMPL (i.e., 4 × 10^3 M^{-1} at 10 °C).

A slight excess of EDTA added to MMPL at 35 °C results in a nearly complete dissociation (estimated K_a < 200 M^{-1}) of PNPM from the protein with an apparent first-order rate constant of 0.071 s^{-1} (Figure 2B). This rate must reflect removal of Mn^{2+} from both S1 and S2 by the excess EDTA. A test for the conformational state of Con A at this point was easily accomplished by the further addition of excess Zn^{2+} and Ca^{2+}. Had the locked protein, PL, reverted to the unlocked conformational state, P, during the removal of Mn^{2+} by EDTA, the free PNPM should have disappeared from solution at a rate of 34 × 10^{-3} s^{-1} (see Table I) upon readdition of Zn^{2+} and Ca^{2+}. Instead, the sugar disappeared immediately upon mixing the two metals (Figure 2C) indicating the protein remained in the locked PL form upon removal of Mn^{2+}. The PNPM binding constant with CMPL prepared by the above procedure is identical with that measured with CMPL prepared by direct equilibration of apo-unlocked Con A with Zn^{2+} and Ca^{2+}. Thus, Zn^{2+} and Ca^{2+} equilibrate rapidly in S1 and S2, respectively, upon their addition to the locked PL conformational form. The addition of Zn^{2+} or Co^{2+} to PL in the absence of Ca^{2+} does not significantly enhance its affinity for PNPM whereas excess quantities of Mn^{2+} or Ca^{2+} alone increase the affinity of PL for PNPM noticeably. This suggests that, unlike Mn^{2+} which binds in both S1 and S2 (Brown et al., 1977), Zn^{2+} and Co^{2+} are capable of binding only in S1 in both the unlocked and locked protein conformational forms.

A similar examination of the ability of the apo-locked conformational form (PL) to bind PNPM was performed at 10 °C. Locked MMPL was prepared at room temperature and added to solution of the sugar at 10 °C. Excess EDTA was added and, after sufficient time for Mn^{2+} removal from PL, the amount of free sugar in solution was measured. The results show that PL has only a slightly smaller affinity for PNPM (3–4 × 10^3 M^{-1}) than does MMPL at 10 °C. Thus, the association constant for sugar binding to PL displays a greater temperature dependence than does CMPL (see Table I).

The ability of the locked conformational form of Con A, CMPL, to retain its sugar binding properties at pH 2.4 was also examined. A mixture of apo-Con A, Mn^{2+}, and Ca^{2+} was allowed to equilibrate at room temperature for several hours. A small volume of this CMPL solution was then injected into a PNPM solution strongly buffered at pH 2.4 with glycine. The rate of sugar release from CMPL was followed polarographically while ESR measurements gave the rate of Mn^{2+} released from the protein. The results are presented in Figure 3. Although data could not be collected during the first 30 min of the polarographic experiment to allow sufficient time for oxygen removal, the data do show 90% release of *both* Mn^{2+} and PNPM after 80 min in the low pH buffer system. This indicates PNPM does bind to CMPL at pH 2.4 but sugar binding is mediated by Mn^{2+} release from the protein. An extrapolation of bound PNPM concentrations back to time equals zero yields an estimated association constant for PNPM binding to CMPL of 3.3 × 0.3 × 10^3 M^{-1}. This indicates the sugar has a factor of 2 smaller attraction for CMPL at pH 2.4

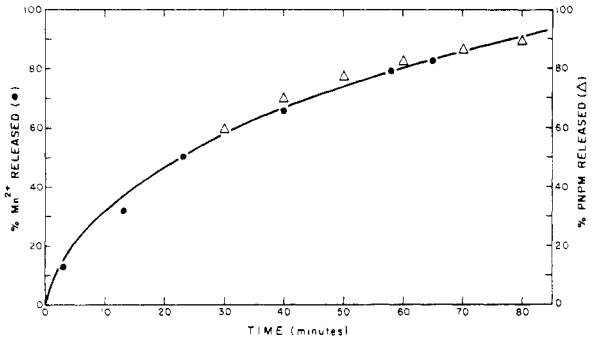

FIGURE 3: Release of manganese and *p*-nitrophenyl α-D-mannopyranoside from the locked conformational form of concanavalin A at pH 2.4 and 25 °C. Concentrated Con A was diluted into 0.1 M glycine buffer to give a solution containing 184 μM protein and 64 μM *p*-nitrophenyl α-D-mannopyranoside. Manganese release was monitored at various times with electron spin resonance spectroscopy and sugar release was followed polarographically.

than at pH 5.6. Our data do not allow us to differentiate between Mn^{2+} dissociation followed by slow PL→ P conversion or concomitant PL→P conversion and Mn^{2+} release as rate determining in this experiment but it is clear that Mn^{2+} (and presumably Ca^{2+}) must be bound to PL at pH 2.4 for the protein to have an appreciable affinity for PNPM.

Discussion

A polarographic study of *p*-nitrophenyl α-D-mannopyranoside in the presence of various metallized derivatives of concanavalin A provides considerable insight concerning the role played by the metals in this protein. Our results are completely consistent with the model presented by Brown et al. (1977), in which the metals Mn^{2+} and Ca^{2+} induce a conformational rearrangement of the protein to a form which is capable of binding simple monosaccharides. This conformational change in solution may be synonymous with the folding of a peptide loop around the two metal sites, as detected by differences in native and demetallized crystal forms (Becker et al., 1976). The polarographic technique shows quite conclusively that neither the apo-"unlocked" protein, P, *or* the metallized "unlocked" protein, CMP, has an appreciable affinity for PNPM ($K_a < 100\ M^{-1}$) over the temperature range of 10–35 °C. The binding of Zn^{2+}, Co^{2+}, or Mn^{2+} into S1 and Ca^{2+} into S2 results in a slow conversion of CMP into CMPL, the "locked" form of the protein which binds sugars. The insensitivity of the first-order rate constants for the CMP→CMPL conversion to the identity of the transition metal ion in S1 (Mn^{2+}, Zn^{2+}, or Co^{2+}) with Ca^{2+} in S2 suggests that ligand field stabilization plays an insignificant role in the conformational transition. The rate constants measured by the polarographic technique are in good agreement with those obtained by Brown et al. (1977) using water proton relaxation data on the Mn^{2+}–Ca^{2+} derivative ($10.5 \times 10^{-3}\ s^{-1}$ at 23 °C and $0.96 \times 10^{-3}\ s^{-1}$ at 5 °C). Thus, removal of water from the Mn^{2+} coordination sphere (as detected by a decrease in the water relaxation rate) during the protein conformational transition parallels the establishment of the sugar binding region. The role played by a metal in S1 may then simply be to partially negate charge repulsions between the rearranging metal ligands Glu-8, Asp-19, and Asp-10 (Becker et al., 1975) at the S1 site thereby lowering the free energy of the active, sugar binding CMPL conformational form.

The activation parameters for the metal ion induced folding process calculated from the data in Table I include an activation energy, $E_a = 25.9 \pm 0.5$ kcal/mol, and an entropy of activation, $\Delta S^{\ddagger} = 17 \pm 1$ eu. The large positive $\Delta S^{\ddagger}$ most probably reflects dehydration of the protein and metals in the activated complex which accompanies the conformational change. A similar, although larger, entropy of activation has been measured for a conformational change in α-chymotrypsin and ascribed to dehydration (Fersht, 1972). Brown et al. (1977) reported an activation energy of 21.7 kcal/mol (derived from rate constants at two temperatures) for the conversion of CMP→CMPL and proposed a cis-trans isomerization of Pro-206 as the rate-determining step in the conformational transition. This proposal was based upon the similarity between their measured activation energy and those found for cis-trans isomerization of X-proline dipeptides (Brandts et al., 1975) and the noted difficulty in fitting the electron density maps to a trans-peptide at Pro-206 (Reeke et al., 1975). Becker et al. (1976) have recently shown that the most significant structural alterations between native and demetallized Con A crystals occur in a tightly folded polypeptide loop (residues 11–23) around the metals and a few nearby side groups, principally Leu-99, Gly-209, Arg-228, and the carboxy terminal group, Asn-237. Although the resolution of the present structural data does not allow a definitive answer to the possible isomerization of the Pro-206 amide bond, it is worth noting that the polypeptide loop which surrounds metals in CMPL also contains three proline residues (Pro-13, Pro-20, and Pro-23). A refinement of the crystallographic data may reveal that one of these isomerizes in the presence of metals and is responsible for the slow structural change which accompanies the "unlocked" to "locked" transformation.

Several individual metals bind in both S1 and S2 metal sites and promote the formation of the active sugar binding structures. These include manganese, lead, cadmium, the trivalent lanthanides, and calcium. The first order rate constants for the conformational conversion in each of these derivatives are very dependent upon the identity of the metal ion which occupies S1 and S2. The results of our studies of the four later metalloforms will be presented elsewhere. The ability of manganese to bind at both sites appears unique among the first row divalent transition metal ions. As the metal ion size requirements for binding in S2 seem more stringent than those for binding in S1 (Sherry et al., 1975), this may simply reflect the larger ionic radius of Mn^{2+} over Co^{2+} and Zn^{2+}. Occupancy of S2 therefore seems to be an absolute requirement for the conformational transition but the rate of the conversion is dependent upon the properties of the metal which occupies this site, i.e., the first-order rate constant for MMP→MMPL is 45 times smaller than the corresponding rate for CMP→CMPL at 35 °C. If one assumes a similar enthalpy of activation for the two rate processes, then a predictably smaller entropy of activation for the MMP→MMPL conversion resulting from less dehydration of Mn^{2+} vs. Ca^{2+} could produce the slower rate for the manganese derivative. However, two pieces of evidence suggest that the final conformational structures of CMPL and MMPL are *not* identical. First, Mn^{2+} ions rapidly dissociate from both metal sites in MMPL ($t_{1/2} \simeq 12$ s at 35 °C) but only slowly from S1 in CMPL ($t_{1/2} \simeq$ several hours at 35 °C). Secondly, the sugar binding constants should be a sensitive function of the conformational state of the protein and, as shown above, the measured PNPM association constants decrease in the order, CMPL > MMPL > PL. Thus, although a mixture of Zn^{2+} and Ca^{2+}, Mn^{2+} and Ca^{2+} or Co^{2+} and Ca^{2+} rapidly equilibrate into their respective sites in PL and added Ca^{2+} rapidly displaces Mn^{2+} from S2 in MMPL (Brown et al., 1977) with no apparent further change in conformation, the decreased affinity of MMPL and PL for PNPM must reflect minor alterations in the structure of the residues near the sugar

binding region.

P. C. Harrington & R. G. Wilkins (private communication) report that Con A at pH 7.2 in the presence of excess Mn^{2+}, excess Ca^{2+}, or a combination of these two metal ions has a similar affinity for the fluorescent sugar, 4-methylumbelliferyl α-D-mannopyranoside even though the glycogen precipitation rates fall in the order, Mn^{2+}-Ca^{2+}-Con A > Mn^{2+}-Con A > Ca^{2+}-Con A. Presumably, Ca^{2+}, like Mn^{2+}, is capable of binding in both S1 and S2 to form the active, locked CCPL structure and, as the data with the fluorescent sugar would suggest, the locked protein conformation may become insensitive to the identity of the metals in S1 and S2 at the higher pH.

The association constants for PNPM binding to CMPL are equivalent when M equals Zn^{2+}, Co^{2+}, or Mn^{2+} and C equals Ca^{2+}. This suggests that the final conformational form of each CMPL metalloform is identical. It should be noted that the association constants determined by the polarographic technique are somewhat smaller than the literature values and the reasons for these differences are not presently clear. However, a search of the literature finds a considerable variation in the reported PNPM-Con A binding constants ($K_a = 0.9 \times 10^4$ M^{-1} at 25 °C, pH 5, Lewis et al., 1976; 3.6×10^4 M^{-1} at 25 °C, Hassing & Goldstein, 1970; 1.5×10^4 M^{-1} at 27 °C, pH 7, Bessler et al., 1974) and this variation may simply reflect minor differences in experimental conditions. The temperature dependence of our equilibrium constants yields an enthalpy change of -7.9 ± 0.4 kcal/mol for the PNPM-CMPL interaction. This value is also considerably more exothermic than the value measured by difference spectroscopy ($\Delta H = -4.76$; Hassing & Goldstein, 1970).

Our experiments with CMPL at pH 2.4 show quite conclusively that Mn^{2+} and PNPM both dissociate from the locked protein conformation at this pH with a $t_{1/2} \simeq 25$ min at 25 °C. We attribute this time dependence to a reversal of the protein conformational change from CMPL to P upon protonation of the metal binding ligands which results in simultaneous dissociation of PNPM, Mn^{2+}, and presumably Ca^{2+}. Thus, removal of the metals at pH 5.6 or 2.4 lowers the free energy of the unlocked conformation thereby favoring this structure. Strong acid conditions apparently lower the activation barrier for the PL→P transition and preclude the formation of the active sugar binding PL structure. Hassing & Goldstein (1970) have presented spectroscopic and equilibrium dialysis evidence for PNPM binding to Con A at pH 2.4 with only a slightly lower association constant than that found at pH 6.5. It is clear from our data that this sugar does bind to the locked form of Con A at pH 2.4 with a slightly smaller affinity (factor of 2) but this process is reversed within 80–90 min of mixing; it remains unclear how equilibrium dialysis data would reflect any measurable amount of sugar binding.

In summary, we have shown that apo-Con A must undergo a metal ion induced protein conformational change before it is capable of binding with sugars at pH 5.6. The conformational transition rate is identical and most rapid when S1 is occupied by Zn^{2+}, Co^{2+}, or Mn^{2+} and S2 by Ca^{2+} and the three resulting metalloproteins have equivalent affinities for PNPM. This conformational rearrangement does not proceed at an appreciable rate in the presence of metal ions which are capable of binding only in S1 but does so at differing rates in the presence of individual metal ions which occupy both S1 and S2. Metal ions may be removed from the active, locked protein structure and the protein temporarily retains its ability to bind with PNPM but with an appreciably lower affinity. The readdition of metal ions into S1 does not alter this affinity but the addition of individual metal ions or a mixture of metal ions which occupy both S1 and S2 increases the affinity of the protein for PNPM. Thus, both the rate of the conformational transition and the resulting active protein-sugar association constants are especially sensitive to the metal ion which occupies S2.

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